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ORIGINAL ARTICLE

Zonulin Antibodies in Iraqi Celiac Disease: A Novel Biomarker Assessment

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ABSTRACT

Background: *Celiac disease (CD) is an autoimmune disorder of the small intestine triggered by gluten (wheat, barley, rye), leading to inflammation, malabsorption, and symptoms such as diarrhea, abdominal pain, and weight loss. Zonulin, a regulator of intestinal permeability, may contribute to CD pathogenesis; zonulin antibodies are reportedly elevated in affected patients.* ***Objectiv****e: The purpose of this study was to assess the association between zonulin antibodies and CD and evaluate their potential as a diagnostic biomarker.* ***Methodology:*** *In this case–control study, 80 CD patients (aged 4–55 years) diagnosed between September 2024 and January 2025 at Imam Hassan Al-Mujtaba Pediatric Teaching Hospital and Imam Al-Hussein Medical City Hospital (Karbala, Iraq) were enrolled alongside healthy controls. Serum anti-tissue transglutaminase (tTG), anti-gliadin antibodies (AGA), and zonulin antibodies were measured by ELISA. Demographic data and zonulin levels were compared using nonparametric statistical tests.* ***Results:*** *CD patients had significantly higher zonulin antibody levels than controls (P = 0.001).* ***Conclusion:*** *Elevated zonulin antibodies in CD patients support a role for zonulin in disease etiology and highlight zonulin antibodies as a promising biomarker for celiac disease.*

INTRODUCTION

Celiac disease (CD) is an autoimmune condition affecting the intestinal tract. It can be caused by exposure to gluten, which is characterized by elevated levels of gliadin peptides. Individuals with genetic susceptibility have digestive enzymes that cannot adequately degrade gliadin, leading to an inflammatory response in the intestines[1].

Gluten is a complicated combination of closely related proteins that dissolve in alcohol while remaining insoluble in water. This substance is distinctive due to its elevated concentration of amino acids, including proline and glutamine. The special resistance to protease degradation in the digestive tract is enhanced by these amino acids[2].

Recent serological screening studies estimate that celiac disease affects approximately 1–2% of the global population, with histologically confirmed cases accounting for about 0.7%[3] . Although the overall prevalence is relatively low, our two study sites—Imam Hassan Al-Mujtaba Pediatric Teaching Hospital and Imam Al-Hussein Medical City—are tertiary referral centers for gastrointestinal disorders serving a broad catchment area in central and southern Iraq; they diagnose an average of 16 new CD cases per month, which enabled us to enroll 80 consecutive patients over the five-month period from September 2024 to January 202. It may manifest with various signs and symptoms at any stage of the patient's life, from weaning to adulthood, for both genders[4] .

Clinical signs in children may encompass oedema, recurrent respiratory infections, vomiting, constipation, increased fecal size, irritability, delayed physical development, and inadequate weight gain. In the adult , a significant proportion of afflicted individuals exhibit no symptoms; nonetheless, some may present with manifestations such as diarrhoea, weight loss, and abdominal distension. Along with intestinal symptoms, patients may also present with extra-intestinal manifestations such as infertility, menstrual irregularities (e.g., amenorrhea or oligomenorrhea), dermatitis herpetiformis, and neurological complications including seizures, ataxia, and peripheral neuropathy[5] .

The pathophysiology of CD encompasses the interplay of genetic and environmental factors. Gluten enteropathy is linked to a widely known hereditary predisposition known as HLA-DQ2 and/or HLA-DQ8[6]. Distinguishing between familial and sporadic celiac disease necessitates an understanding of this genetic uniqueness[7].

Celiac disease is diagnosed through a combination of methods, including biopsies, clinical evaluation, and blood tests. Detecting autoantibodies, such as anti-tissue transglutaminase (tTG) and anti-gliadin antibodies (AGA), in blood tests is often considered a convenient diagnostic approach[8]. Cytokines are minimal proteins that are not structural, characterized by small molecular

weight. They help the immune system communicate between cells by coordinating the functions of different types of cells in various areas of the body to make a unified immune response. These cytokines participate in the advancement of chronic inflammatory conditions typically linked to CD[9].

Zonulin is a protein family that influences the tight junctions of the small intestine, hence affecting its permeability. In celiac disease, the immune system responds due to the breakdown of tight junctions, increased permeability, and the infiltration of gluten peptides through the epithelial barrier[10].

Zonulin antibodies are immunoglobulins produced by the host immune system in response to elevated zonulin levels and increased intestinal permeability. Upon exposure to gluten in susceptible individuals, zonulin release from enterocytes disrupts tight junctions, allowing macromolecules to traverse the epithelial barrier. The ensuing immune activation not only targets gliadin and tissue transglutaminase but can also generate antibodies against zonulin itself. Several studies have reported significantly higher zonulin antibody titers in patients with active celiac disease compared to healthy controls or those with non-celiac gastrointestinal disorders, correlating with disease severity and mucosal damage[11].

In adult celiac patients who have adhered to a gluten-free diet for over a year. The scientists demonstrated a strong correlation between intestinal permeability and zonulin levels. Regrettably, a limited number of patients attained complete normalization of these metrics after adhering to a gluten-free diet; the majority did not, perhaps due to continued gluten intake[12].

Therefore, the aim of this study was to evaluate serum zonulin antibody levels in patients with biopsy-confirmed celiac disease compared to healthy controls, to examine their relationship with established serological markers (anti-tTG, AGA) and clinical parameters, and to assess the potential of zonulin antibodies as a non-invasive diagnostic and monitoring biomarker for celiac disease.

METHODOLOGY

Case control study included 80 participants with clinically diagnosed CD, ranging in age from 4 to 55 years. The group consisted of 18 males and 62 females, recruited from two hospitals in Karbala, Iraq: Imam Hassan Al-Mujtaba Pediatric Teaching Hospital and Imam Al-Hussein Medical City Hospital. A control group of 48 healthy adults (10 males, 38 females) was also included, between September 2024 and January, 2025.

Demographic Characteristics

Sex , age and BMI demographics were obtained directly from patient records. Data were classified into age brackets (< 10,10–19 ,20–29, 30–39 and ≥ 40 years) to evaluate the distribution among CD patients and controls.

Sample collection

Sociodemographic data were collected from celiac disease patients and healthy controls via structured, questionnaire-based interviews. Phlebotomy was performed by trained personnel at the hospital's Aspiration Unit, drawing approximately 4 mL of whole blood into Vacutainer gel tubes. Each tube was labeled with a unique barcode and transported to the Immunology Unit for processing. Samples were centrifuged at 3,000 rpm for 20 minutes; about 1 mL of the resulting serum was then aliquoted into labeled Eppendorf tubes and stored at –20 °C until analysis. Additionally, it included healthy controls. Serum anti-tissue transglutaminase (tTG), anti-gliadin antibodies (AGA), and zonulin antibody levels were determined in both control and celiac disease patients using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY). Continuous variables are presented as mean ± SD and were first assessed for normality with the Kolmogorov–Smirnov test. Comparisons of normally distributed continuous variables between celiac patients and controls were made using the independent-samples t-test; for non-normally distributed data, the Mann–Whitney U test was applied. Categorical variables are expressed as frequencies and percentages and were compared using the chi-square test.To assess the diagnostic performance of zonulin antibodies, receiver operating characteristic (ROC) curve analysis was performed, calculating the area under the curve (AUC), optimal cutoff value, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

As shown in table 1 The study population was divided into five age groups: under 10, 10-19, 20-29, 30-39, and over 40 years. The overall age distribution of the participants was as follows: 12.5% were under 10, 18.8% were 10-19, 28.9% were 20-29, 26.6% were 30-39, and 13.3% were over 40. Within CD patients group, the age breakdown was: 13.8% under 10, 32.0% aged 10-19, 23.8% aged 20-29, 23.8% aged 30-39, and 16.2% over 40. The control group exhibited a distribution of: 10.4% under 10, 12.5% aged 10-19, 37.5% aged 20-29, 31.2% aged 30-39, and 8.3% over 40.Statistical analysis revealed no significant difference in age distribution between the CD patients and the control group (P = 0.208).

Table 2 and Figure 1 presents the gender distribution; the study included 28 males (21.9%) and

100 females (78.1%). Patients with celiac disease included 18 men (22.5%) and 62 females (77.5%), whereas the control group included 10 males (20.8%) and 38 females (79.2%). The patients with celiac disease and the control group were compared. The findings indicated no significant change. The gender ratio between patients and control participants was determined to be 0.825.

Table 3 shows the mean zonulin levels of 0.93 ± 0.23 in celiac disease individuals and 0.62 ± 0.21 in the healthy control group. The mean levels were elevated in celiac disease patients compared to the healthy controls, with a statistically significant difference (P = 0.001).

Table 1: Age Groups Distribution Comparison Between Patients and Controls.

Age	**Celiac disease** N=80	**Healthy control** N=48	**Total**	**p-value**
Mean ± SD	**25.53 ± 7.39**	**24.81 ± 6.62**		0.772 † NS
< 10 years, n%	**11 (13.8%)**	**5 (10.4%)**	16 (12.5%)	0.208 ¥ NS
10-19 years, *n*%	**18 (32.0%)**	**6 (12.5%)**	24 (18.8%)	
20-29 years, *n*%	**19 (23.8%)**	**18 (37.5%)**	37 (28.9%)	
30-39 years, *n*%	**19 (23.8%)**	**15 (31.2%)**	34 (26.6%)	
≥ 40 years%	**13 (16.2%)**	**4 (8.3%)**	17 (13.3%)	

n: number of cases; SD: standard deviation; †: Independent T test; ¥: Chi-square test; NS: non-significant at P < 0.05

Table 2: Comparison of patients and control group sex distribution

Study groups	**Sex**		**Total**	**p-value**
	Male	Female		
Celiac disease patients	18 (22.5%)	62 (77.5%)	80	0.825 ¥ NS
Control	10 (20.8%)	38 (79.2%)	48	
Total	28 (21.9%)	100 (78.1%)	128	

¥: Chi-square test; NS: not significant at P > 0.05

Table 3: Zonulin Abs level in patients and healthy controls.

Zonulin Abs	**Celiac Disease Patients (n = 80)**	**Healthy Controls (n = 48)**	**p-value**
Mean ± SD	0.93 ± 0.23	0.62 ± 0.21	0.001**†
Range	0.41 – 3.00	0.10 – 2.59	

n: number of cases; SD: standard deviation; †: Independent T test; **: significant at P > 0.05

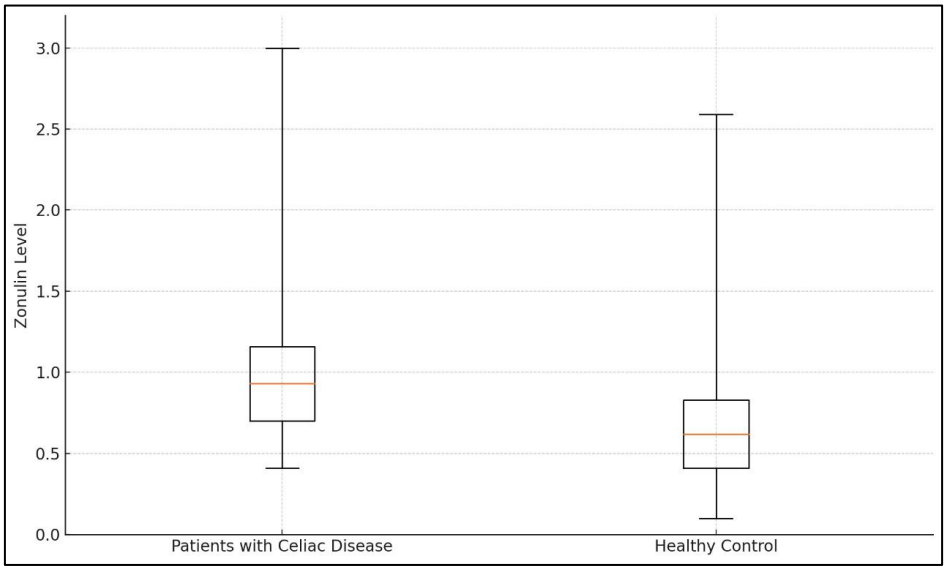

Fig. 1: The means level of Zonulin Abs in patients and control groups

Table (4) show the Zonulin cutoff value was > 0.73-fold with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and area under curve of 71.2%, 70.8%, 80.3%, 59.6% and 0.714 (0.609- 0.818). The present results indicates Zonulin is considered as an acceptable diagnostic marker.

Table 4: Sensitivity and specificity of Zonulin Abs level (> 0.73-fold) in Celiac disease

Zonulin Abs level	patients *n* = 80	Healthy control *n* = 48
> 0.73	57 (%)	14 (%)
< 0.73	23 (%)	34 (%)
Sensitivity %	71.2 %	
Specificity %	70.8 %	
PPV %	80.3 %	
NPV %	59.6%	
AUC (95% CI)	0.714 (0.609- 0.818)	

CI: Confidence interval, AUC: Area under the curve

DISCUSSION

Celiac disease (CD) is an autoimmune condition of the small intestine induced by the consumption of gluten, a protein present in wheat, barley, and rye. It is caused by an abnormal immunological reaction to gluten, resulting in inflammation and injury to the small intestine's lining; thus, it decreases nutrient absorption[13]. Although serological and biopsy-based studies now place its prevalence at about 1% worldwide, celiac disease was long underdiagnosed and regarded as a rare, primarily pediatric disorder due to limited awareness and testing; it is now recognized across all age groups[14].

Our results indicates no significant difference in age distribution between patients and controls. The results are consistent with previous studies by Khatoon, *et al.* [15],Majeed [16], who found that the same percentage of patients in their 20s had the trait (37.8% and 37.93%, respectively).

Contrary to previous beliefs that celiac disease primarily affected children, it is now known to be an autoimmune disease that can occur at any age[17]. The greater recognition of celiac disease in adults is attributed to its ability to manifest at any stage of life, coupled with the development of highly reliable blood tests.

The rising incidence and the shifting demographics of CD are in part explained by better recognition and screening in adults. In fact, with age at diagnosis, the antibody titers decrease, and histological damage is less marked. It is common to find adults without villous atrophy showing only an inflammatory pattern in duodenal mucosa biopsies; this lower clinical, analytical, and histological expressiveness in adults makes their diagnosis more complex than in pediatric forms[18].

Our study indicates a greater prevalence of females in both the patient and control groups, with affected females comprising 77.5% while males 22.5%, in CD patients This difference corresponds with data from a study conducted in Finland by Koskinen, *et al.* [19] which indicated that a lower percentage of males (36.8%) were diagnosed with coeliac disease compared to females (63.2%)

Autoimmune diseases are more prevalent in females than in males due to the presence of XX sex chromosomes in women, compared to the XY sex chromosomes in men. A higher percentage of immune-related and immune regulatory genesare located on the X chromosome, and these genes are responsible for facilitating and enhancing immunological responses in the body[20,21]. The presence of two X chromosomes results in an "overdose" of X-linked genetics, thereby predisposing females to autoimmune diseases[22].

The current study revealed a significant increase zonulin antibodies in individuals with celiac disease (0.93 ± 0.23) compared to a control group (0.62 ± 0.21; p=0.001), a similar pattern of results was obtained byRabiee, *et al.* [11],Sturgeon and Fasano[23] as also reported in other studies. Zonulin overexpression and defective tight junctions are recognized features of autoimmune conditions, including celiac disease.

Notably, celiac disease is unique in having gliadin as a known environmental trigger, which interacts with intestinal cells to break down tight junctions. This disruption leads to increased zonulin production, a peptide that regulates tight junctions and contributes to heightened intestinal permeability[24].

Clinicians should be aware of patients' elevated serum zonulin levels, which suggest increased intestinal permeability (IP). Given that zonulin is the only known regulator of IP, future therapies for chronic pain syndromes may focus on repairing the intestinal barrier by blocking the zonulin pathway[25]. Furthermore, new research indicates that zonulin has promise as an early predictor of celiac disease, underlining its potential for detection and treatment[10].

In terms of diagnostic accuracy, zonulin levels in the current study exhibited a sensitivity of 71.2% and a specificity of 70.8%. These findings are in agreement with previous studies, DaFonte, *et al.* [10], which found that elevated serum zonulin was associated with increased intestinal permeability and a higher risk of developing celiac disease, reporting a sensitivity and specificity exceeding 80%.

CONCLUSIONS

This study demonstrates that patients with celiac disease exhibit a significantly elevated level of zonulin

antibodies compared to the control group. The findings support a strong association between zonulin and the pathophysiologic mechanisms of celiac disease. This suggests that zonulin antibodies could be a useful biomarker for the early detection of celiac disease.

Ethics Approval

The study protocol was reviewed and approved by the Institutional Review Board of Imam Hassan Al-Mujtaba Pediatric Teaching Hospital and the Ethics Committee of Imam Al-Hussein Medical City (Approval No. IRB/2024/015). All procedures were conducted in accordance with the Declaration of Helsinki and local regulations for human subject research.

Consent to Participate

Written informed consent was obtained from all adult participants. For children (aged < 18 years), consent was provided by a parent or legal guardian.

Consent for Publication

Not applicable—no identifying individual-level data or images are included in this manuscript.

Conflicts of Interest

The authors declare that they have no competing interests.

Publication Statement

This manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

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